JCI The Journal of Clinical Investigation

Selective Binding Site for $[^3H]$Prostacyclin on Platelets

Adelaide M. Siegl, … , J. Bryan Smith, Melvin J. Silver

J Clin Invest. 1979;63(2):215-220. https://doi.org/10.1172/JCI109292.

Research Article

Prostacyclin (PGI_2) is the most potent, naturally occurring inhibitor of platelet aggregation known. To determine whether PGI_2 is bound by platelets, high specific activity $[9^3H]PGI_2$ was synthesized by iodination and subsequent base treatment of the labeled precursor $[9\text{-}^3H]$prostaglandin (PG)$F_{2\alpha}$ methyl ester. Binding experiments were performed at room temperature with normal citrated human platelet-rich plasma that contained $[^{14}C]$sucrose or $[^{14}C]PGF_{1\alpha}$ as an internal marker for the extracellular space. Binding of $[^3H]PGI_2$ plateaued within 2 min and this bond radioactivity could be displaced rapidly by excess nonradioactive PGI_2. Scatchard analysis of concentration-dependent binding yielded a hyperbolic plot which appeared to be caused by the existence of two classes of binding sites. The higher affinity class has a dissociation constant of 12.1±2.7 nM and a capacity of 93 (±21)sites per platelet. The lower affinity class had a dissociation constant of 0.909±.236 μM and a capacity of 2,700±700 sites per platelet. The relative ability of PGI_2, PGE_1, PGE_2, and 6-keto-$PGF_{1\alpha}$ to displace $[^3H]PGI_2$ initially bound to the higher affinity class of sites were 100:5:<0.3: <0.3. These relative abilities parallel the relative potencies of these compounds as inhibitors of ADP-induced platelet aggregation in vitro. However PGD_2, which is more potent than PGE_1 as an inhibitor of aggregation, did not displace bound $[^3H]PGI_2$. The higher affinity binding site for PGI_2 appears to be the specific receptor […]

Selective Binding Site for [^{3}H]Prostacyclin on Platelets

ADELAIDE M. SIEGL, J. BRYAN SMITH, and MELVIN J. SILVER, *Cardeza Foundation and Department of Pharmacology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107*

K. C. NICOLAOU, *Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19174*

D. AHERN, *New England Nuclear, Boston, Massachusetts 02118*

ABSTRACT Prostacyclin (PGI_2) is the most potent, naturally occurring inhibitor of platelet aggregation known. To determine whether PGI_2 is bound by platelets, high specific activity [9-^{3}H]PGI_2 was synthesized by iodination and subsequent base treatment of the labeled precursor [9-^{3}H]prostaglandin (PG)$F_{2\alpha}$ methyl ester. Binding experiments were performed at room temperature with normal citrated human platelet-rich plasma that contained [^{14}C]sucrose or [^{14}C]$PGF_{1\alpha}$ as an internal marker for the extracellular space. Binding of [^{3}H]PGI_2 plateaued within 2 min and this bond radioactivity could be displaced rapidly by excess nonradioactive PGI_2. Scatchard analysis of concentration-dependent binding yielded a hyperbolic plot which appeared to be caused by the existence of two classes of binding sites. The higher affinity class has a dissociation constant of 12.1 ± 2.7 nM and a capacity of 93 (± 21)sites per platelet. The lower affinity class had a dissociation constant of $0.909 \pm .236$ μM and a capacity of $2{,}700 \pm 700$ sites per platelet. The relative ability of PGI_2, PGE_1, PGE_2, and 6-keto-$PGF_{1\alpha}$ to displace [^{3}H]PGI_2 initially bound to the higher affinity class of sites were 100:5:<0.3: <0.3. These relative abilities parallel the relative potencies of these compounds as inhibitors of ADP-induced platelet aggregation in vitro. However PGD_2, which is more potent than PGE_1 as an inhibitor of aggregation, did not displace bound [^{3}H]PGI_2. The higher affinity binding site for PGI_2 appears to be the specific receptor through which PGI_2 exerts its effect on platelets.

INTRODUCTION

Until recently, the most potent inhibitor of platelet aggregation known was prostaglandin (PG)E_1[1] (1). In 1974, PGD_2 was found to be more potent as an inhibitor of human platelet aggregation although it was relatively inactive on platelets obtained from other species (2). In 1976, Moncada et al. (3) discovered an even more potent but unstable inhibitor of platelet aggregation which was produced from prostaglandin endoperoxides by an enzyme present in blood vessels. They proposed that the continuous synthesis of this substance protects arterial walls against the deposition of platelet thrombi (4). The structure of this inhibitor has been determined and it is now known as prostacyclin (PGI_2) (5). Cultured endothelial cells possess the capacity to synthesize PGI_2 (6, 7). It inhibits the aggregation of platelets from all species examined (8, 9).

PGE_1, PGD_2 and PGI_2 have been shown to activate adenylate cyclase and increase cyclic (c)AMP levels in human platelets (1, 10–12). Tateson et al. (12) demonstrated that PGI_2 at 10 nM caused an increase in cAMP levels equivalent to five times the basal level, whereas it required 1 μM PGD_2 to acheive this increase. The stimulation of adenylate cyclase by PGI_2 argues for its interaction with a specific membrane-bound receptor system similar to that involved in the β-adrenergic system (13). We describe here the properties of a binding site for PGI_2 on intact platelets, which appears to be its specific membrane receptor.

METHODS

PGI_2 was prepared by the method of Nicolaou et al. (14). For the preparation of [^{3}H]PGI_2, 8 μg of [9-^{3}H]$PGF_{2\alpha}$ methyl ester (10–12 Ci/mmol sp act) was dissolved in 70 μl methylene chloride and reacted overnight with 16 μg of iodine in the presence of 0.2 mg potassium carbonate at −10°C. The product, $PGF_{2\alpha}$-methyl ester iodide, was purified by thin-layer chromatography (5% methanol in diethyl ether). Treatment of the purified intermediate with 60 μl sodium ethoxide in the presence of 160 μl 95% ethanol at 75°C for 1 h removed both the iodine atom and the ester group to yield [9-^{3}H]PGI_2, which was stable because of the basic conditions involved in its preparation. Thin-layer chromatography of [9-^{3}H]PGI_2 (chloroform: methanol; acetic acid; water; 90;9;1.0;0.6) indicated 95% purity. Other prostaglandins were a gift from Dr. John Pike, The Upjohn Co., Kalamazoo, Mich.

This work has been published in abstract form in 1978. *Fed. Proc.* 37: 260.

Received for publication 31 July 1978 and in revised form 23 October 1978.

[1] *Abbreviations used in this paper;* cAMP, cyclic AMP; PG, prostaglandin; PGI_2, prostacyclin; PRP, platelet-rich plasma.

Blood from normal volunteers who had not taken drugs for 10 d was drawn into 0.1 vol 3.8% trisodium citrate and centrifuged at 180 g for 15 min. Platelet-rich plasma (PRP) was removed and the platelet count was determined with a Coulter Counter (Coulter Electronics Inc., Hialeah, Fla.) Platelet aggregation was measured at 37°C in an aggregometer (Chrono-log Corp., Havertown, Pa.) with 20 μM ADP as the aggregating agent. Prostaglandins were added in ethanol up to a maximum concentration of 0.5% vol/vol.

In binding experiments, 0.2 μCi/ml [U-^{14}C]sucrose (3.6 Ci/mol sp act) or 0.05 μCi/ml [1-^{14}C]PGF$_{1\alpha}$ (45 Ci/mol sp act; New England Nuclear, Boston, Mass.) was added to the PRP as an internal marker for the extracellular space. Appropriate amounts of [^{3}H]PGI$_2$ were added to the PRP and the sample was incubated at room temperature for a given time. Prostaglandins were added in ethanol up to a maximum concentration of 0.5% vol/vol. Incubation was terminated by centrifuging 1-ml samples in an Eppendorf microfuge (Brinkman Instruments, Inc., Westbury, N. Y.) at 15,000 g for 2 min. The supernate was removed rapidly with a Pasteur pipette. The tube was inverted, allowed to drain for 5 min, and the pellet was removed with a cotton swab. The pellets and samples of the supernates were oxidized in a Packard 306 Oxidizer (Packard Instrument Co., Inc., Downers Grove, Ill.). The [^{3}H]H$_2$O and [^{14}C]CO$_2$ were determined by liquid scintillation counting. Efficiency of combustion was 99% and counting efficiencies were determined by combustion and liquid scintillation counting of known amounts of radioactive standards. Bound [^{3}H]PGI$_2$ per 10^8 platelets was determined after correction for background, extracellular space, and platelet count. In some experiments, bound [^{3}H]PGI$_2$ was expressed as femtomoles per 10^8 platelets using its known specific activity after correction for counting efficiency.

RESULTS

Effect of PGI$_2$ on platelet aggregation. Prostacyclin caused 50% inhibition of aggregation induced by 20 μM ADP at a final concentration of 3.4 nM. PGD$_2$ was ≅ 7% as effective causing 50% inhibition of aggregation at 50 nM and PGE$_1$ was only 3.5% as effective as PGI$_2$. PGE$_2$, PGF$_{1\alpha}$, and 6-keto-PGF$_{1\alpha}$ were <0.15% as effective as PGI$_2$ because they caused no detectable inhibition of aggregation at a concentration of 50 μM.

Time-course of binding and displacement. Incubation of PRP with a low concentration of [^{3}H]PGI$_2$ (9 nM) for increasing lengths of time indicated that PGI$_2$ was bound by platelets. The amount of [^{3}H]PGI$_2$ bound plateaued within 2 min and remained at approximately this level for the next 10 min (Fig. 1, solid line). Similar results were obtained with a high concentration of [^{3}H]PGI$_2$ (118 nM) (data not shown). When a 100-fold excess of unlabeled PGI$_2$ was added 5 min after the 9 nM [^{3}H]PGI$_2$, there as a rapid displacement of the bound radioactivity. The displacement was essentially complete after 2 min and the amount displaced accounted for 80% of the bound radioactivity (Fig. 1, open circles). In all subsequent experiments the PRP was incubated with [^{3}H]PGI$_2$ for 5 min to ensure that equilibrium had been established.

Concentration dependence. PRP was incubated with concentrations of [^{3}H]PGI$_2$ ranging from 4.5 to

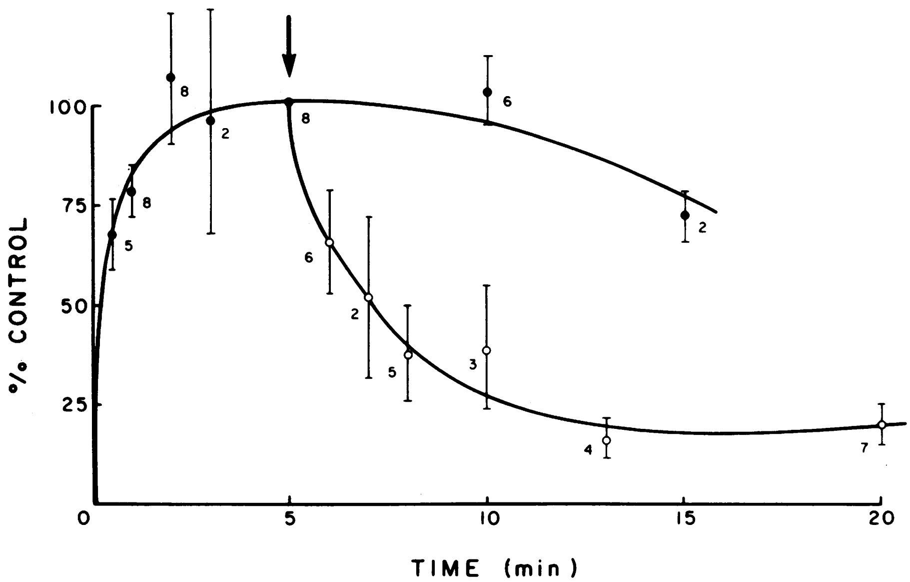

FIGURE 1 Time-course of binding and displacement. PRP was incubated with [^{3}H]PGI$_2$ for a given time and binding was measured with [^{14}C]sucrose as the internal marker as described in Methods. Results are plotted as the percent of control disintegrations per minute bound (●) against the time of incubation. 100% control disintegrations per minute bound is the disintegrations per minute bound per 10^8 platelets at 5 min for each experiment. With 9 nM PGI$_2$ this ranged from 253 to 309 dpm/10^8 platelets. The arrow indicates that 1 μM unlabeled PGI$_2$ was added 5 min after the [^{3}H]PGI$_2$ (○). Each point represents the mean of two–eight experiments (±SEM). Platelet counts were between 2.64 and 4.88 × 10^8 platelets/ml.

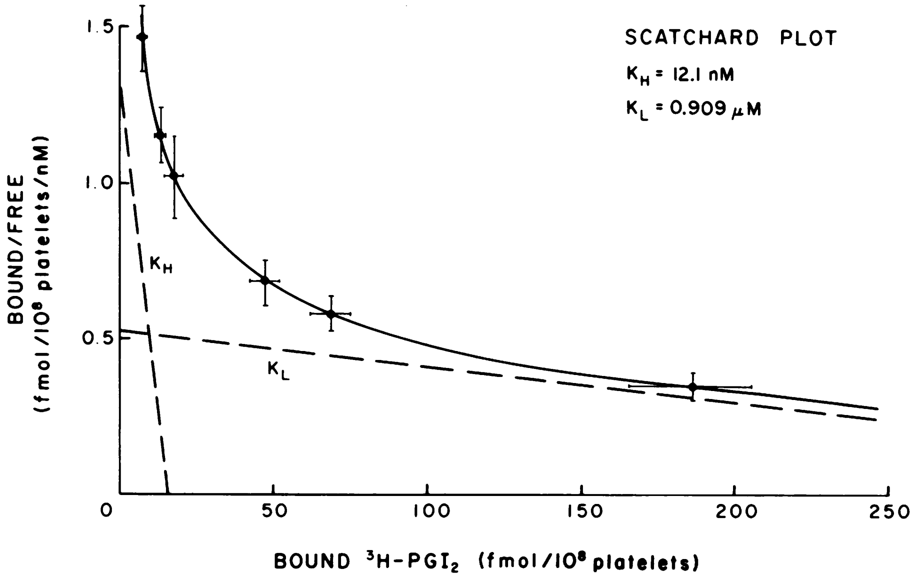

FIGURE 2 Scatchard analysis of concentration-dependent binding. PRP was incubated with [^{3}H]PGI$_2$ in concentrations from 4.5 to 518 nM and binding was measured with [^{14}C]sucrose as the internal marker. Each point is plotted ±SEM. The smooth curve represents the hyperbolic plot and the dotted lines were used to obtain K_H and K_L. Each point represents the mean of 14 determinations on seven subjects. With an unpaired Student t test each point was significantly different from its neighbors at a P level of from <0.05 to <0.001.

518 nM. Scatchard analysis of the data is shown in Fig. 2. A curvilinear plot was obtained when values were plotted over the complete concentration range. This curve was a hyperbola, because a straight line (correlation coefficient, 0.996) was obtained when the same data was plotted on double log paper. The two asymptotes to this hyperbola (the product of which describes the hyperbola) were derived by iterative geometric construction (Fig. 2, dashed lines). It was assumed that the hyperbola reflected the binding of [^{3}H]PGI$_2$ to two independent sites with different affinities and the asymptotes were used to derive the dissociation constants for each of these sites (15). This analysis yielded a higher affinity, low-capacity site with a dissociation constant (K_H) of 12.1±2.7 nM and a capacity of 93±21 sites per platelet. The lower affinity, high capacity site had a dissociation constant (K_L) of 0.909±0.236 μM and a capacity of ≅ 2,700±700 sites per platelet.

Specificity of prostacyclin binding. Several prostaglandins were tested for their ability to displace [^{3}H]-PGI$_2$ from platelets. In Fig. 3 it can be seen that unlabeled PGI$_2$ causes displacement of radioactivity at low concentrations. PGE$_1$ was the next most potent compound, whereas PGE$_2$ produced only a slight amount of displacement and PGD$_2$ caused essentially no displacement. The concentrations of PGI$_2$, PGE$_1$, PGE$_2$, and PGD$_2$ required to displace 50% of the bound [^{3}H]PGI$_2$ were 0.3, 6, and <100 μM, respectively. Therefore, the relative affinities were 100:5:<0.3:<0.3 for PGI$_2$, PGE$_1$, PGE$_2$,, and PGD$_2$. The ability of 6-keto-PGF$_{1\alpha}$, PGF$_{2\alpha}$, or PGF$_{1\alpha}$ to displace bound [^{3}H]-PGI$_2$ was also determined (Fig. 3). Slight displacement was observed with PGF$_{1\alpha}$ and 6-keto-PGF$_{1\alpha}$, whereas essentially no displacement was observed with PGF$_{2\alpha}$. The affinity for 6-keto-PGF$_{1\alpha}$ was <0.3% relative to PGI$_2$.

Comparison of extracellular space determinations with [^{14}C]sucrose or [^{14}C]PGF$_{1\alpha}$. Table I illustrates an experiment in which the binding of [^{3}H]PGI$_2$ was determined with either [^{14}C]sucrose or [^{14}C]PGF$_{1\alpha}$ to correct for the extracellular space. It can be seen that the values obtained are identical. Furthermore, an essentially identical rate of displacement of [^{3}H]PGI$_2$ by nonradioactive PGI$_2$ was obtained in one experiment with either [^{14}C]sucrose or [^{14}C]PGF$_{1\alpha}$ as the internal marker. In another experiment, with equilibrium conditions and [^{14}C]PGF$_{1\alpha}$ as the internal marker, two binding sites were obtained on Scatchard analysis. These had dissociation constants of 12.4 nM and 0.93 μM almost identical to those obtained in the seven experiments with [^{14}C]sucrose shown in Fig. 2.

DISCUSSION

To be relevant to the action of a hormone the binding of a radioactive ligand should be saturable, reversible, and specific, and should be comparable in time-course and concentration to the effect produced (16).

To determine the binding of a hormone to intact cells

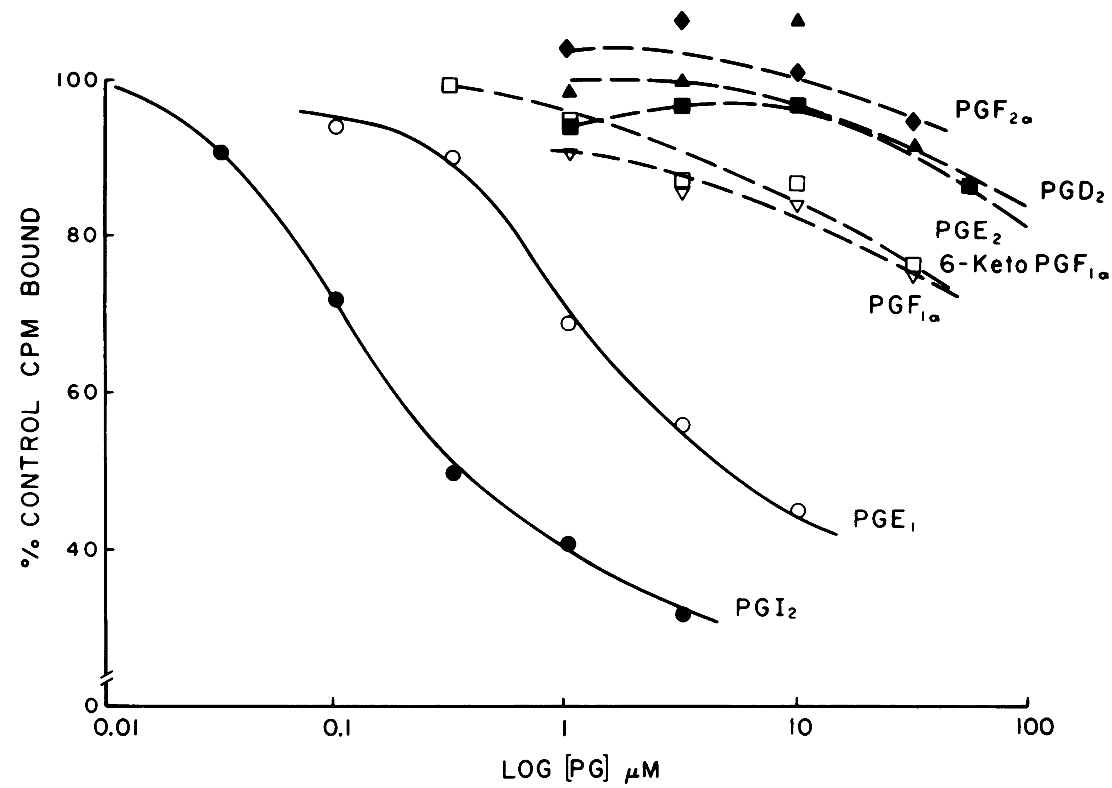

FIGURE 3 Ability of prostaglandins to displace [^{3}H]PGI_2 initially bound to the higher affinity site. PRP was incubated for 5 min with 4.5 nM PGI_2 thereby achieving 20% saturation of the high binding site and <2% saturation of the low affinity site. Varying concentrations of unlabeled prostaglandins were added, the incubation was continued for an additional 10 min, and binding was determined with [^{14}C]sucrose as the internal marker. Relative ability PGI_2 ●; PGE_1 ○; PGE_2 ■; PGD_2 ▲; $PGF_{1\alpha}$ ▽; 6-keto-$PGF_{1\alpha}$ □; $PGF_{2\alpha}$ ◆ to displace bound [^{3}H]PGI_2. Each point represents the mean of at least six values obtained in three different experiments. 100% control counts per minute was equal to 157 ± 21 dpm/10^8 platelets and was a measure of the [^{3}H]PGI_2 bound after 15 min. Platelet count was $3.78\pm0.78 \times 10^8$ platelets/ml.

it is usual to employ a second radioactive compound which neither penetrates the cells nor is bound to them as a marker for extracellular space. In most of our experiments we used [^{14}C]sucrose as this marker. However, we found that [^{14}C]$PGF_{1\alpha}$ could be used equally well as the marker (Table I) and therefore we justified all conclusions reached with sucrose as the marker by also using [^{14}C]$PGF_{1\alpha}$.

As shown in Fig. 1, the binding of PGI_2 by platelets was rapid and 80% of the binding was complete by 1 min. The effects of PGI_2 on aggregation and increases in platelet cAMP occur within this same time (11, 12), suggesting that binding is associated with almost immediate activation of adenylate cyclase. The rapid displacement of bound [^{3}H]PGI_2 by unlabeled PGI_2 (Fig. 1) shows that binding was reversible and suggests that PGI_2 is bound at an extracellular site. It previously has been shown that E- and F-type prostaglandins do not equilibrate across membranes in the absence of an active transport system (17). This is consistent with our findings that the plasma spaces determined with [^{14}C]sucrose and [^{14}C]$PGF_{1\alpha}$ were identical (Table I).

The concentration-dependent aspects of PGI_2 binding were complex as a hyperbolic plot was obtained on Scatchard analysis (Fig. 2). We assumed that this data reflected the binding of [^{3}H]PGI_2 to two independent sites with different affinities. An alternative explanation could be that negative cooperativity is involved (18).

The dissociation constant (12.1 nM) of the higher affinity binding site for PGI_2 is of the same order of magnitude as that found by other investigators studying prostaglandin binding in several other biological systems (16, 19) although it is fourfold higher than the value obtained for 50% inhibition of platelet aggregation (3.4 nM). However, a direct constant is a measure of the direct physical interaction of PGI_2 with its receptor, whereas the effective dose for 50% inhibition of aggregation reflects complex interactions involv-

TABLE I
Determination of $[^3H]PGI_2$ Binding with Two Different Markers for Plasma Space

	Plasma space marker	
	$[^{14}C]PGF_{1\alpha}$	$[^{14}C]$sucrose
^{14}C-measurements		
In 20 μl supernate, *cpm*	1,375±40	3,447±80
In pellet, *cpm*	200±27	524±45
Calculated plasma space, μl	2.85±0.39	3.01±0.27
$[^3H]PGI_2$ measurements		
In 20 μl supernate, *cpm*	811±40	881±40
In pellet, *cpm*	241±25	250±18
In plasma space, *calculated cpm*	120±16	137±12
Bound by platelets, *calculated cpm*	121±4	113±12

Each number represents the mean of three values±SD corrected for background. These were obtained in a single experiment in which the equilibrium binding of $[^3H]PGI_2$ at 6 nM was tested with either $[^{14}C]PGF_{1\alpha}$ or $[^{14}C]$sucrose as the marker for the extracellular space. There were 4.99×10^8 platelets/ml. Other details are given in Methods.

ing elevation (and sometimes reductions) of cAMP (20). As shown in Fig. 2, at 1.8 nM PGI_2, only about 12 molecules of PGI_2 are bound per platelet, yet presumably this produces a sufficient biochemical stimulus to inhibit aggregation. The concentration of PGI_2 required to cause a half-maximal increase in cAMP in intact platelets has been reported as 0.2 μM (12) or 40 nM (11). That reported to half maximally stimulate adenyl cyclase in platelet membranes was 28 nM (12). These values differ by a factor of from 2 to 10 from the dissociation constant we obtained for the higher affinity site. These differences may reflect the fact that cyclic AMP measurements over short time periods are not equilibrium measurements and do not accurately measure PGI_2 activation of adenyl cyclase.

The structural specificity of prostaglandin binding was investigated by testing the ability of several unlabeled prostaglandins to displace $[^3H]PGI_2$ initially bound to the higher affinity site on platelets (Fig. 3). The concentration of PGI_2 required to displace 50% of this bound radioactivity was 0.3 μM. As expected, this value lay between the dissociation constants for the lower and higher affinity sites because some of the radioactivity displaced from the higher affinity sites reequilibrated with the large number of lower affinity sites during the 10-min incubation period. PGE_1 also displaced bound $[^3H]PGI_2$ although it was only 5% as active as PGI_2 itself. The remainder of prostaglandins, including PGD_2 were <0.3% as effective as PGI_2. PGI_2 is unstable at neutral pH and spontaneously converts to 6-keto-$PGF_{1\alpha}$. Because 6-keto-$PGF_{1\alpha}$ was almost incapable of displacing bound radioactivity, it is unlikely that the binding of 6-keto-$PGF_{1\alpha}$ was a factor in our measurements.

With the exception of PGD_2, the results of these displacement experiments correlate well with the relative potencies obtained in the aggregation experiments. PGD_2 did not displace bound PGI_2 but was approximately twice as active as PGE_1 as an inhibitor of aggregation (7% as effective as PGI_2). PGD_2 also increases cAMP in platelets and its potency, relative to PGE_1 and PGI_2, correlates well with its ability to inhibit aggregation (12). Previous to the discovery of PGI_2, Mills and Macfarlane (10) suggested that PGE_1 and PGD_2 may act on different receptors on platelets because of subtle differences noted in the time-course of increases in cAMP produced by these two prostaglandins. Subsequently, based on measurements of platelet cAMP, they suggested that PGE_1 and PGI_2 act on the same receptor, whereas PGD_2 acts on another (21, 22). The results of the binding experiments, therefore, support the conclusions drawn from these biochemical studies.

MacDonald and Stuart (23) originally reported that PGE_1 is bound by intact platelets. This work recently has been extended by Schafer et al. (24) who showed that $[^3H]PGE_1$ is bound by human platelet membranes. Scatchard analysis of their data also indicated the existence of two binding sites. Furthermore, consistent with the idea of a common receptor, they found that either PGE_1, PGI_2, or PGE_2 could displace bound $[^3H]PGE_1$.

In conclusion, we have described a high-affinity binding site for PGI_2 on platelets that fulfills most of the necessary criteria for a specific receptor. Our results indicate that PGD_2 does not occupy this receptor. It will be of interest to determine whether platelets of patients with thrombotic tendencies have a diminished number of binding sites for PGI_2.

ACKNOWLEDGMENTS

We thank Dr. D. E. Macfarlane for helpful discussions.

This work was partially supported by National Institutes of Health grant HL 14890.

REFERENCES

1. Smith, J. B., and D. E. Macfarlane. 1974. Platelets. *In* The Prostaglandins. P. W. Ramwell editor. Plenum Publishing Corporation, New York. 2: 293–343.
2. Smith, J. B., M. J. Silver, C. M. Ingerman, and J. J. Kocsis. 1974. Prostaglandin D_2 inhibits the aggregation of human platelets. *Thromb. Res.* 5: 291–299.
3. Moncada, S., R. Gryglewski, S. Bunting, and J. R. Vane. 1976. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature (Lond.).* **263:** 663–665
4. Gryglewski, R. J., S. Bunting, S. Moncada, R. J. Flower,

and J. R. Vane. 1976. Arterial walls are protected against deposition of platelet thrombi by a substance (Prostaglandin x) which they make from prostaglandin endoperoxides. *Prostaglandins.* **12:** 685–713.

5. Johnson, R. A., P. R. Morton, J. H. Kinner, R. R. Gorman, J. R. McGuire, F. F. Sun, N. Whittaker, S. Bunting, J. A. Salmon, S. Moncada, and J. R. Vane. 1976. The chemical characterization of prostaglandin X (prostacyclin). *Prostaglandins.* **12:** 915–928.
6. Weksler, B. B., A. J. Marcus, and E. A. Jaffee. 1977. Synthesis of PGI_2 (prostacyclin) by cultured human and bovine endothelial cells. *Proc. Natl. Acad. Sci. U. S. A.* **74:** 3922–3926.
7. MacIntyre, D. E., J. D. Pearson, and J. L Gordon. 1978. Localization and stimulation of prostacyclin production in vascular cells. *Nature (Lond.).* **271:** 549–551.
8. Nicolaou, K. C., W. E. Barnette, G. P. Gasic, R. L. Magolda, W. J. Sipio, M. J. Silver, J. B. Smith, and C. M. Ingerman. 1977. Rapid and easy preparation of prostacyclin *Lancet.* **I:** 1058–1059.
9. Moncada, S., J. R. Vane, and B. J. R. Whittle. 1977. Relative potency of prostacyclin, prostaglandin E_1 and D_2 as inhibitors of platelet aggregation in several species. *J. Physiol. (Lond.).* **273:** 2P–4P.
10. Mills, D. C. B., and D. E. Macfarlane. 1974. Stimulation of human platelet adenylate cyclase by prostaglandin D_2. *Thromb. Res.* **5:** 401–412.
11. Gorman, R. R., S. Bunting, and O. V. Miller. 1977. Modulation of human platelet adenylate cyclase by prostacyclin (PGX). *Prostaglandins.* **13:** 377–388.
12. Tateson, J. R., S. Moncada, and J. R. Vane. 1977. Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platelets. *Prostaglandins.* **13:** 389–397.
13. Levitski, A., N. Sevilla, and M. L. Steer. 1976. The regulatory control of β-receptor dependent adenylate cyclase. *J. Supramol. Struct.* **4:** 365–378.
14. Nicolaou, K. C., W. E. Barnette, G. P. Gasic, R. L. Magolda, and W. S. Sipio. 1977. Simple, efficient synthesis of prostacyclin (PGI_2). *J. Chem. Soc. Chem. Commun.* 630–631.
15. Klotz, I. M., and D. L. Hunston. 1971. Properties of graphical representations of multiple classes of binding sites. *Biochemistry.* **10:** 3065–3069.
16. Rao, G. V., 1976. Discrete prostaglandin receptors in outer cell membranes of bovine corpora lutea. *Adv. Prostaglandin Thromboxane Res.* **1:** 247–258.
17. Bito, L. F. 1975. Are prostaglandins intracellular, transcellular or extracellular autocoids. *Prostaglandins.* **9:** 851–855.
18. De Meyts, P. 1976. Cooperative properties of hormone receptors in cell membranes. *J. Supramol. Struct.* **4:** 241–258.
19. Schillinger, E., and G. Prior. 1976. Characteristics of prostaglandin receptor sites in human uterine tissue. *Adv. Prostaglandin Thromboxane Res.* **1:** 259–264.
20. Haslam, R. J., and S. Taylor. 1971. Role of cyclic 3′5′ adenosine monophosphate in platelet aggregation. *In* Platelet Aggregation. J. P. Caen, editor. Masson et Cie, Paris. 85.
21. Mills, D. C. B., D. E. MacFarlane, and K. C. Nicolaou. 1977. Interaction of prostacyclin (PGI_2) with the prostaglandin receptors on human platelets that regulate adenylate cyclase activity. *Blood.* **50:** 247.
22. Mills, D. C. B. 1977. Platelet aggregation and the adenylate cyclase system. *In* Platelets and Thrombosis. D. C. B. Mills and F. E. Pareti, editors. Academic Press, Inc., New York. 63–70.
23. MacDonald, J. W. D. and R. K. Stuart. 1974. Interaction of prostaglandins E_1 and E_2 in regulation of cyclic AMP and aggregation in human platelets: evidence for a common prostaglandin receptor. *J. Lab. Clin. Med.* **84:** 111–121.
24. Schafer, A. I., B. Cooper and R. D. Handin. 1978. Characterization of platelet prostaglandin receptor. *Clin. Res.* **26:** 356A (Abstr.)